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SUPPLEMENT ARTICLE

Treatment of early life status epilepticus: What can we learn from animal models?

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Summary

Treatment of status epilepticus (SE) in infants and children is challenging. There is a recognition that a broad set of developmental processes need to be considered to fully appreciate the physiologic complexity of severe seizures, and seizure outcomes, in infants and children. The development and use of basic models to elucidate important mechanisms will help further our understanding of these processes. Here we review some of the key experimental models and consider several areas relevant to treatment that could lead to productive translational research. Terminating seizures quickly is essential. Understanding pharmacoresistance of SE as it relates to receptor trafficking will be critical to seizure termination. Once a severe seizure is terminated, how will the developing brain respond? Basic studies suggest that there are important acute and long-term histopathologic, and pathophysiologic, consequences that, if left unaddressed, will produce long-lasting deficits on the form and function of the central nervous system. To fully utilize the evidence that basic models produce, age- and development- and model-specific frameworks have to be considered carefully. Studies have demonstrated that severe seizures can cause perturbations to developmental processes during critical periods of development that lead to life-long deficits. Unfortunately, some of the drugs that are commonly used to treat seizures may also produce negative outcomes by enhancing Cl^--mediated depolarization, or by accelerating programmed cell death. More research is needed to understand these phenomena and their relevance to the human condition, and to develop rational drugs that protect the developing brain from severe seizures to the fullest extent possible.

KEY WORDS: Status epilepticus, Neonatal seizures, Antiepileptic drugs, Brain development, Epilepsy.

Experimental models of status epilepticus (SE) in the young have provided important information regarding the short- and long-term consequences of severe seizures. Most of them are adapted from adult models of SE,[1] and comparisons of the young to the adult are complicated by a host of developmental factors[2] including, but not limited to, differences in metabolic rate,[3,4] shifting developmental gene expression patterns,[5] sequential receptor roles over developmental time,[6] and unsynchronized periods of neurogenesis, synapse formation, and synapse elimination,[7] leading to selective windows of normal and pathologic[8] programmed cell death in distinct cell populations.[9] Given that modeling human development in animals is fraught with uncertainties,[10] translational conclusions require considerable caution but can reveal some basic principles.[11]

Recent clinical and basic studies have highlighted the negative impact of SE in the young on brain and behavior, including epileptogenesis,[12,13] long-term changes in brain imaging,[14] as well as cognitive function.[15,16] Although some controversies remain, basic and clinical studies have

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Key Points

- At all ages tested (from postnatal day 7 to adulthood in the rat), some long-lasting seizures cause neuronal injury and leave chronic sequelae
- Even in the absence of neuronal death, status epilepticus can adversely affect brain development
- The drugs used to treat seizures can also have deleterious effects on the developing brain, particularly in neonates and infants
- More research is needed to understand the mechanisms and improve treatment of early life seizures, and to identify drugs that do not interfere with brain development

converged.[7,17–20] Basic studies have shown that the mechanisms of seizure induction, and maintenance, can change both over developmental time and during individual seizures. Clinical studies have confirmed the adverse effects of seizure activity on brain development and outlined the great diversity of syndromes with unique clinical presentation of SE, often linked to specific genetic defects.[21–23]

In an ideal scenario, basic models of developmental SE would have clear translational value in a manner that would inform acute treatments and the long-term care of patients. Imitative models try to mimic the human illness as closely as possible (the best model of a cat is another cat, and preferably the same cat), whereas reductionist models isolate one feature of the human illness to understand its mechanism and design a treatment. Both types can be useful for different purposes, and if they reproduce key clinical hallmarks predictably, efforts to understand, and treat, the disease process can move forward. Here we evaluate data derived from several models of SE in the developing brain, with the hope that continued research in this field will lead to more options and better treatments in the clinic.

Pathophysiology of SE

Critical periods of brain development

There are many "critical periods" of brain development. This concept can be traced experimentally back to Hans Spemann at the turn of the twentieth century, and it was related directly to synaptic activity and connectivity, by the work of Hubel and Wiesel.[24] Cell and synapse survival are dependent on synaptic activity in a region- and developmental time-specific manner. Cortical visual networks that are disconnected from visual input during a critical period do not develop normally, and cannot regain full function, if reconnected after the end of that period. Competition for neurotrophins that are released in an activity-dependent manner (some of which are known to be modified by seizures[25]), during periods of synaptogenesis and synapse stabilization, seems to be crucial to that process.

These principles also apply to disease processes, as illustrated by the effects of thyroid hormone on development: thyroid hormone is required between prenatal day 3 and postnatal day 5–10 (P5-10) for development of the auditory system, and between birth and P20 for cerebellar development. Restoration of thyroid function after P10 no longer cures deafness, and thyroid hormone replacement after P21 fails to restore cerebellar function, Purkinje cell branching, or granule cell migration, showing that once a critical period of central nervous system development is bypassed, it cannot be retraced.[26] Such precise timing is not available for the effects of seizures on brain development, but experimental data suggest that critical periods often coincide with periods of rapid developmental change in the affected neuronal population. For example, repeated seizures during P9-P18 in rat pups reduce myelin accumulation permanently,[27] and around P20 they permanently modify the field and function of hippocampal place cells.[28]

The context in which seizures occur varies dramatically with age. The brain of the neonate and infant has a very low metabolic rate and few excitatory circuits, so that some forms of damage take longer to develop.[3] Maturation follows a progressive curve,[29] peaking during childhood in a brain with more synapses, richer excitatory networks with proportionally more CA^{2+}-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors,[30,31] and a higher metabolic rate than the adult brain, and these changes are associated with high neuronal vulnerability but significant potential for recovery. Adult and elderly brains display a slightly lower metabolic rate, high vulnerability, and a lower adaptive and recovery potential. We have to distinguish the infant from the child, since the brain environment dramatically alters seizure patterns and their consequences.

Pharmacoresistance

We have very effective mechanisms to stop seizures, yet they sometimes fail and lead to self-sustaining, pharmacoresistant SE. Early pioneers like Trousseau understood that this implies a change in the properties of the brain. Seizure-induced increases in extracellular potassium[32] and in intracellular chloride[33] facilitate depolarization and reduce hyperpolarization, and depletion of key peptides[34,35] tilts the balance toward excitation. Inhibitory modulators, such as adenosine, may become depleted extracellularly during extended seizures.[36,37] The most critical change in the transition from single seizures to SE in adults may be the internalization and temporary inactivation of synaptic γ-aminobutyric acid A $(GABA)_A$ receptors ($GABA_AR$s) in hippocampus and other brain regions,[38,39] which accounts, in part, for the failure of GABAergic inhibition[40] and for the poor response of established SE to benzodiazepines and other GABAergic drugs.[41] Seizure-induced receptor trafficking also increases synaptic *N*-methyl-D-aspartate (NMDA) and AMPA receptors,[42,43]

enhances excitatory drive, and may play an important role in the maintenance phase of SE. These changes suggest that the standard treatment consisting of benzodiazepine monotherapy followed by other antiepileptic drugs, then anesthesia,[44] addresses only the $GABA_AR$ changes and leaves changes in glutamate receptors untreated. Indeed, in experimental models of adult SE, combinations of $GABA_AR$ agonists with glutamate receptor antagonists are synergistic[45,46] and are far more effective than benzodiazepines, especially when treatment is late.[47]

There are few studies to tell us if these mechanisms apply to the immature brain. In postnatal day 28 (P28) rats, self-sustaining SE and pharmacoresistance are present,[48] but at P14, perforant path stimulation delivered for 16 h consecutively failed to make seizures self-sustaining,[49] and pharmacoresistance to diazepam was not seen in P14 rat pups in lithium-pilocarpine seizures[48] or in P21 rats in soman-induced seizures.[50] Treatment with glutamate receptor antagonists has been successful in some models[51] but has not been investigated systematically in immature animals. NMDAR antagonists display age-specific toxicity, which precludes their use in the very young.[52] Given that $GABA_ARs$ are underrepresented, and glutamate receptors are overrepresented, in the immature brain (postinfancy), we need to understand whether similar transitions occur during SE in the highly connected young brain, and in the very young, which do not typically display seizure generalization and self-sustaining SE.[49]

Seizure circuits

In adult, free-running animals, SE tends to become self-sustaining (SSSE) and to continue for hours after the ictogenic stimulus is withdrawn. This is true of seizures induced by chemical[48,53,54] as well as electrical stimulation.[55,56] Robust electrographic activity that remains confined to a pathway in the young,[49] would typically spread and generalize in the adult.[57] This may lead to the different behavioral seizure phenotypes, with a paucity of convulsive activity commonly reported in very young animals,[58–60] and likely also results in regional or focal vulnerabilities in the immature brain. This "electroclinical dissociation" has been discussed in relation to treatment in neonates.[61] Global brain scans may, or may not, detect isolated lesions,[15] but the reports of long-term sequelae[7,17,62] suggest that neuronal death and related pathologic changes can result directly from SE in the young. Understanding the conditions that are critical for the transition from stimulus-bound seizures to SSSE may help us to understand at what point SE becomes intractable and brain damaging, and how to prevent these consequences.

SE and neuronal injury/death

Does SE damage the immature brain?

Autopsies show widespread neuronal death in children dying from prolonged febrile convulsions or SE but cannot tell us whether seizures caused neuronal injury. Key studies reported associations between mesial temporal sclerosis and early SE or extended febrile convulsions.[63–65] These findings supported the pursuit of animal models that could explain the human data in the immature brain. Adult animal models of SE using seizure induction with kainate,[66] high-dose pilocarpine,[67] lithium-pilocarpine,[68] or electrical stimulation,[69] produce neuronal injury, including hippocampal sclerosis and secondary epileptogenesis. Several models have been adapted for use in immature animals.[49,58,70–73] Studies in young animals using convulsants that reliably produced SE and seizure-induced damage in adult rats, reported differences from mature animals in seizure-induction thresholds, mortality, electrographic and behavioral seizure phenotype, and in histopathologic outcomes. It is clear that the impact of SE in young animals has to be evaluated in a model-specific framework.

Chemoconvulsant models of SE in the first weeks of life

Kainic acid (KA), when delivered at doses comparable to those in adult animals has a mortality rate as high as 90% in 15-day-old rats. This complication can lead to 2 obvious problems. One problem is the selection bias of surviving animals that experience less severe seizures, and another problem is that by adjusting doses downward to improve survival, less severe seizures are produced. When KA seizures are experienced by rats 2 weeks of age, the animals progress through electroencephalography (EEG) and behavioral seizure stages phenotypically different from adults.[58,74,75] These differences make direct comparisons to adult animals tenuous. The fact that immature rabbits experience hippocampal damage following KA-induced seizures suggests that model-specific effects need to be considered.[76]

Pilocarpine given at a high dose reliably produces SE and widespread damage, including severe hippocampal sclerosis, in the adult brain.[67] When moderate and high doses were given to rats in the first weeks of life, complex behavioral alterations did not manifest reliably within the first week, and only some of the animals showed signs recognized as limbic seizures at P12.[59] The seizures became more robust and consistent with the adult pattern of EEG and behavior by the third week of life, but lethality also increased. Damage was seen in some of these animals, but fewer than would be anticipated by the adult model.

On first analysis, the studies looking for histopathologic effects and sequelae of SE in the immature brain seemed to show a resistance to damage.[58,59] These basic data have been used to argue that the immature brain is selectively resistant to seizure-induced death,[10,77] and that clinicians might better serve their patients by stopping convulsive activity and focusing on the underlying cause of the SE rather than the seizures per se.[78] This concept that severe extended seizures in the young were benign was challenged by a number of studies using different models of SE.

Lithium pretreatment allows lower doses of pilocarpine to induce SE. The reduction of peripheral side effects at lower dose reduces mortality. This model produces robust SE in P12-P15, and reliably shows widespread damage that predominates in the hippocampus[72] (Fig. 1 A–B), and in extratemporal lobe areas.[70,79] It also produces secondary epileptogenesis with spontaneous recurrent seizures.[72] Histologic evidence of damage in immature animals has been confirmed by elevated serum levels of neuron-specific enolase, a marker of neuronal death.[80] In addition, immature rabbits show hippocampal and extrahippocampal lesions resulting from lithium-pilocarpine seizures[73] showing that seizure-induced damage can be demonstrated in multiple models (Fig. 1D).

It is important to note that recent studies using P7-P10 rat pups, which are developmentally similar to human neonates, and which also have been assumed to be refractory to seizure-induced neuronal injury, display neuronal death in the absence of hypoxemia and long-term sequelae following SE. P10 rat pups show acute and chronic damage and epileptogenesis following 30 min of SE.[81] P7 animals show widespread acute neuronal death in a model of severe SE[82] (Fig. 1C).

SE induced by electrical stimulation

A model of perforant path stimulation (PPS) in 2-week-old rats was adapted from an adult model in anesthetized rats.[83] In the P14-P16 rats, the long-lasting anesthetic urethane could not be used because it caused enhanced damage in areas undergoing programmed cell death within the piriform cortex.[84] The pups were fitted with chronic head implants, and the perforant path was stimulated unilaterally in free-moving animals. This seizure-like stimulation induced selective loss of GABAergic hippocampal interneurons, as well as principal cells, in the stimulated hippocampus, and left the contralateral hippocampus intact, demonstrating that neuronal death was the result of seizure activity and not due to hypoxia or other systemic factors. There was an early phase of apoptotic death in the border between the hilus and dentate granule cells (Fig. 1E), followed by losses in the hilus proper and principal cells. Of interest, even with continuous long-term stimulation, the animals did not enter SSSE as older animals subjected to the same conditions typically do.[56] This demonstrated that even in the developing brain, sustained focal seizure-like discharge can damage an overactive neural network.

Hyperthermia-induced seizures

Hyperthermia-induced seizures, used to model febrile seizures, show clear long-term impacts such as enhanced hippocampal excitability, and the occurrence of spontaneous seizures in some animals.[85] Hippocampal and extrahippocampal injury has been reported in this model using silver staining, but no gross detectable cell drop out could be detected chronically, although unbiased stereology was not used. A study using magnetic resonance imaging (MRI) did show hippocampal abnormalities and associated learning and memory deficits in some animals.[15] A significant portion of pups with long-lasting seizures displayed increased hippocampal T2 signal acutely, and atrophy, chronically, on MRI. MRI studies performed in children with prolonged febrile seizures[86] show acutely increased hippocampal T2 evolving into hippocampal atrophy in a sizable subset of patients. It is notable that experimental studies suggest that hyperthermia may be a major contributor to seizure-associated neuronal injury in the young[81] (Fig. 2).

Adverse effects of seizures in the reported absence of neuronal death

Not all basic studies investigating the histopathologic consequences of recurrent seizures or SE in the young have reported observable, or permanent, cell damage in limbic structures that is typically detected using conventional methods in mature animals,[712,7,87] although ruling out a small amount of cell death is difficult.[88] Recovery may be a major factor in some neuronal populations. Cell injury, or loss, may be compensated for by ongoing neurogenesis (developmental and/or seizure-induced).[89] Synapse proliferation and survival during critical periods of development are dependent on trophic support, and seizure-induced upregulation of trophic factors such as brain-derived neurotrophic factor[25] may also compensate by preventing signs of atrophy. However, seizures can adversely affect brain development even in the absence of neuronal death and atrophy, and they do so in a strikingly age-specific fashion.

Repeated, single, flurothyl-induced seizures,[60,90] or a single bout of SE[4] induced in infant rats, can inhibit DNA and protein synthesis, irreversibly reduce brain weight, delay behavioral milestones,[91] and reduce seizure thresholds, in the absence of histologic cell loss.[92] Seizures occurring after the peak of brain mitotic activity do not reduce brain cell number, but curtail the build-up in myelin[27] and in synaptic markers,[93] suggesting a reduction in dendritic and/or axonal growth. Seizures throughout brain development inhibit protein synthesis in the regions of the brain most actively involved in seizure activity.[94] Recurrent flurothyl seizures, delivered over the course of several days, produce aberrant mossy fiber sprouting, a long-lasting reduction in seizure thresholds, and spatial memory deficits.[16,92,95] Alteration of developmental regulation of synaptic circuits in the hippocampus by early life seizures has been implicated in long-term cognitive deficits.[7]

Many investigators have considered the deleterious effects of developmental SE to be the result of transient seizure-mediated perturbations in developmental sequences.[2,7] Genetic and developmental programming dysregulation,[5] disturbances in neurogenesis,[89] circuit building, and pruning[96] are all believed to contribute to many of the adverse outcomes that have been described following SE. But so too would cell loss generated by

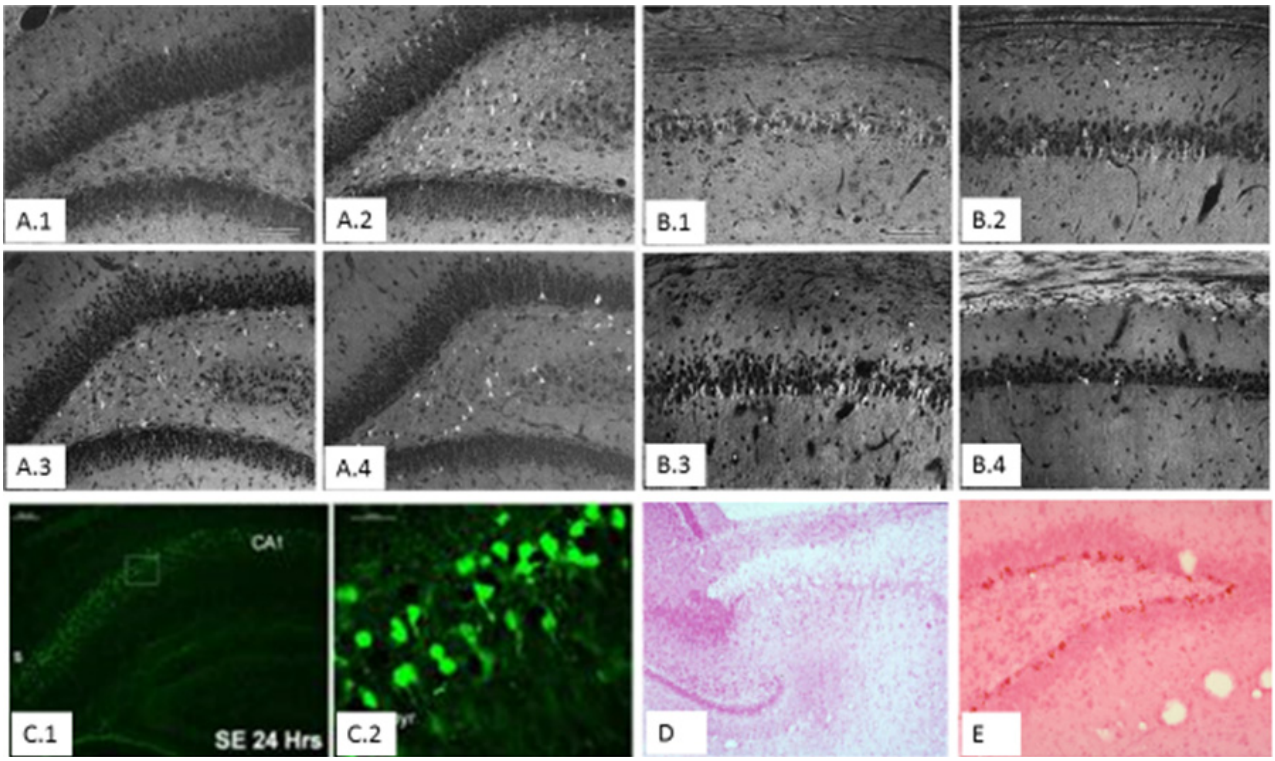

Figure 1.

Evidence of SE-associated cell death in the immature brain from several models. **A1–4**, Age-dependent variation in dentate granule cells and hilar interneurons damaged after lithium-pilocarpine seizures in rats pretreated with lithium (**A1**). Limited eosin fluorescence is seen in a 2-week-old pup 24 h after SE (**A.2**). A 3-week-old pup shows extensive damage to the hilar and outer granule cells (**A.3**). Damaged hilar cells are also visible in a 4-week-old and an adult rat (**A.4**). **B.1–4**, CA1 of a 2-week-old (**B.1**), 3-week-old (**B.2**), and 4-week old pup (**B.3**) shows a large number of eosinophilic cells (hematoxylin and eosin), whereas the CA1 of a mature rat (**B.4**) has fewer injured neurons. Scale bars for A and B, 100 μm (figures taken from reference #63). **C.1–2**, Severe SE results in neuronal injury in both dorsal and ventral CA1. Images of Fluoro-Jade B in CA1 show eosinophilia 24 h after seizure (SE24Hrs). **C.2** is a higher magnification of the boxed area on the image on the left. (figure taken from Reference #73). **D**, Histologic lesions in normoxemic rabbits after lithium-pilocarpine SE. Lesions in CA1 were seen in all animals that had severe seizures. The most severe case of hippocampal damage is shown with total destruction of the CA1 and CA3 pyramidal cell layers. **E**, In situ end-labeling 2 h after perforant path stimulation in the immature rat. Labeled nuclei were seen in cells located within the inner layer of the granule cells bilaterally and cells within the hippocampus that contained labeled nuclei were eosinophilic.

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severe seizures.[13,14,97,98] One possibility is that there is not a strict dichotomy between pathophysiology with damage and pathophysiology without damage, and that the insults to the immature brain caused by SE lie on a continuum of cell death and injury. Conceptual rejection of this dichotomy has been posited in the clinical literature as well.[99–101] Considering SE-induced neuronal damage in the young on a continuum helps to reconcile conflicting data in the literature.[1,11,102] For example, using an identical lithium-pilocarpine model and the same rat strain, one group of researchers reported chronic hippocampal neuronal loss in only a limited subset of animals that experienced hours of SE and went on to develop spontaneous seizures,[103] whereas another group using sensitive staining techniques that detect acute neuronal injury found damage in 100% of animals.[79]

Seizures, drugs, and neuronal injury

In addition to its vulnerability to seizures, the immature brain is also vulnerable to some of the drugs used to treat them.[104] Both NMDA blockers and GABA agonists have been shown to induce apoptosis during critical periods of development, with different neuronal populations showing vulnerabilities at different times in development.[8,84,104] The vulnerable period varies with the drug, but in rodents covers mostly neonates and infants. This exacerbation of damage may not be limited to $GABA_A$ agonists like benzodiazepines[105,106] or barbiturates[107] and NMDA blockers but may extend to many commonly used anticonvulsants. These drugs can cause damage at high dose, and recent data suggest that at lower dose, some of them may have the capacity to worsen seizure-induced damage and sequelae[106] (Fig. 3). Anesthetics may similarly cause neuronal apoptosis at

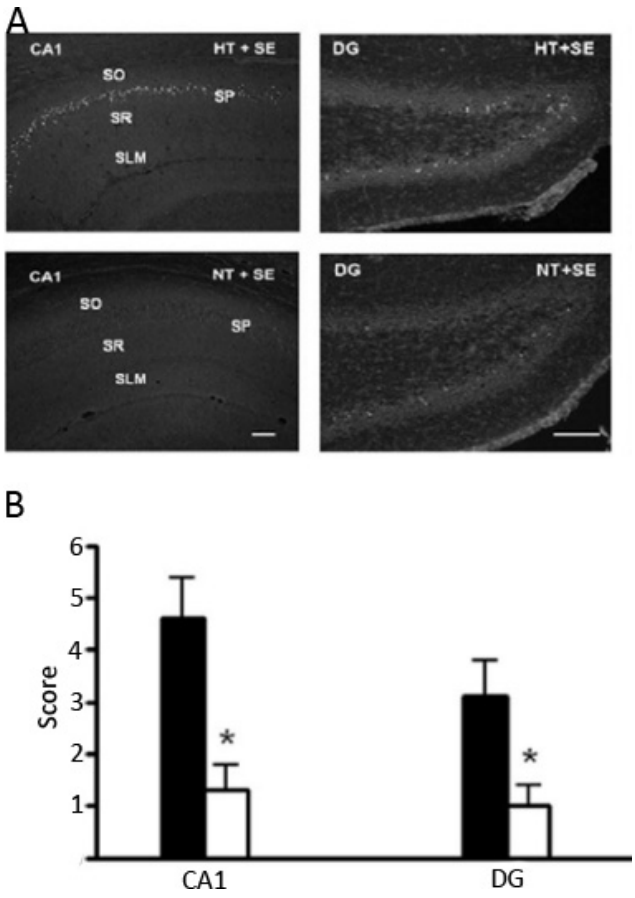

Figure 2.

Acute neuronal injury after 30 min of lithium-pilocarpine SE in P10 rat pups. **A**, At 24 h, both hyperthermia SE (HT + SE, temperature during SE 39 ± 10°C) animals and normothermia SE animals (NT + SE, temperature during SE 35 ± 10°C) showed Fluoro-Jade B–positive neurons in CA1 and DG of hippocampus. **B**, The severity of the neuronal injury was significantly higher in HT + SE animals. Severity score is shown on the y-axis. Abbreviations: SO, stratum oriens; , stratum radiatum; SLM, stratum lacunosum moleculare; SP, stratum pyramidale; CA1, cornus ammonis region; DG, dentate gyrus. Scale bar 100 μm. Values are means ± SEM, *t*-test, $p < 0.05$.

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specific periods of early brain development.[108] There are no solid clinical data to tell us whether this vulnerability applies to humans, although some clinical data seem to support the potential negative effects of early treatment with specific antiepileptic drugs (AEDs).[109,110]

This "developmental drug-induced apoptosis" is a major unresolved issue, since many of the drugs that cause it are used clinically, for example, in the treatment of neonatal seizures, where the drugs of choice are GABAergic, these could depolarize neurons and remove the magnesium block of NMDARs, and could cause, or aggravate, neuronal injury. Drug-induced apoptosis could preferentially target immature cells, so that the behavioral expression of that damage would not appear until much later in development. Recent evidence suggests that the standard drugs recommended for treatment of neonatal seizures by the International League Against Epilepsy (ILAE) aggravate seizure-induced neuronal injury in P7 rats.[106] Furthermore, drugs that do not cause developmental drug-induced apoptosis[104,111,112] are available but are not used as first-line therapy. Further research into the mechanisms of this drug-induced apoptosis, and its human relevance, if any, is urgently needed.

Elevated glutamate release caused by excessive neuronal discharge leads to unregulated Ca^{2+} entry into cells that can cause long-term cellular dysregulation and excitotoxic brain damage.[113,114] The Ca^{2+} -permissive NMDA receptor has been implicated in triggering both necrosis and apoptosis during SE, and both NMDA and non-NMDA channels participate in seizure-induced cell death.[115,116] Blocking the NMDA receptor with the open channel blocker MK-801 protects interneurons and principal cells from PPS-induced damage and preserves GABA-mediated inhibition that would otherwise be lost. Treatment with MK-801 protects neurons without necessarily stopping seizure discharge,[117] so NMDA blockade may be a reasonable neuroprotection strategy in SE that is super-refractory[118] in the adult, but NMDA blockers can induce widespread neuronal loss in neonates and infants.[104,119] For that reason, NMDA blockers are not clinically usable in the very young.

Basic studies reveal intriguing developmental differences between NMDA channel participation in the seizure circuits of the young and mature hippocampus that are unrelated to apoptosis. The NMDA open channel blocker MK-801, administered during perforant path stimulation in P15-P16 free-moving rats, produced an immediate loss of frequency-dependent paired-pulse inhibition (in the absence of brain damage).[120] This drug-induced effect is not seen in the adult model. In fact, NMDA blockade can restore paired-pulse inhibition in the damaged adult brain following PPS.[121]This striking difference between the immature and mature system suggests that there are unique glutamatergic receptor profiles[6] and/or circuits that modulate hippocampal inhibition in the young brain that are lost, or reorganized, later in development. These data also suggest that pharmacologic manipulation of inhibitory circuits may be a route to control highly excitable immature hippocampal circuits.

It is interesting that NMDA blockade can protect from seizure-induced apoptotic cell death in some areas of the immature brain, while at the same time causing an enhancement of apoptosis in other regions. NMDA blockade produces partial protection of both hilar interneurons (presumably both inhibitory interneurons and excitatory "mossy cells") during PPS. Subtypes of GABAergic neurons containing peptide transmitters are lost in both the immature and adult brain following PPS, in a pattern similar to that found in resected tissue from humans with temporal lobe epilepsy.[122] The loss of paired-pulse inhibition at the

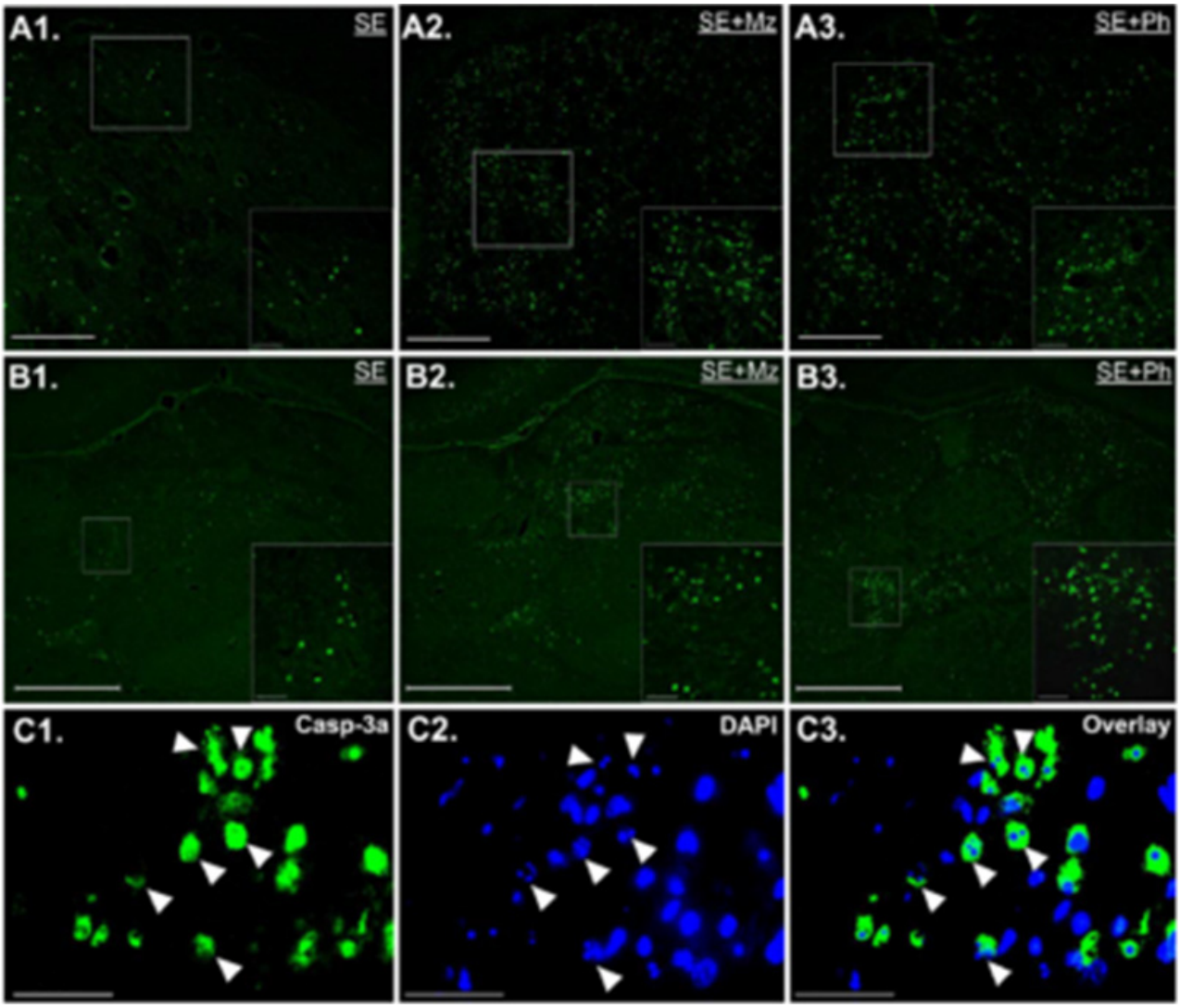

Figure 3.
Effect of midazolam (Mz) or phenobarbital (Ph) treatment on status epilepticus (SE)–associated neuronal injury in caudate putamen and thalamus, and mechanism of thalamic injury in P7 rat pups. SE was induced with high-dose lithium (5 mEq/kg, i.p.) and pilocarpine (320 mg/kg, s.c.). Pups remained normoxemic during SE. Treatment was given after 10 min of SE. **A**, Fluoro-Jade B (FJB) staining in caudate putamen of (**A1**) SE, (**A2**) SE + Mz, and (**A3**) SE + Ph pups with a higher magnification of the boxed area on the bottom right of each image. SE induces neuronal injury throughout caudate, and this injury is exacerbated following midazolam or phenobarbital treatment. **B**, Images of FJB staining in thalamus of (**B1**) SE, (**B2**) SE + Mz, and (**B3**) SE + Ph pups. Both midazolam and phenobarbital treatment increase FJB cell distribution throughout various thalamic nuclei. Scale bars: (**A**) long bars = 200 μm and short bars = 20 μm; (**B**) long bars = 500 μm and short bars = 50 μm. (**C**) Images of overlay of active caspase-3 (Casp-3a) immunoreactivity (green) and 4,6-diamidino-2-phenylindole (DAPI; blue) staining in thalamus of SE + Mz pups. On the left, high magnification shows distribution of caspase-3a–immunoreactive cells in ventromedial thalamus of an SE + Mz pup; in the middle, high-magnification images show that caspase-3a–immunoreactive cells have fragmented nuclei indicative of neuronal cell death; on the right, this overlay shows that many of these caspase-3a–immunoreactive cells have fragmented nuclei, suggesting a caspase-dependent form of cell death. Scale bars = 50 μm.
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outset of the experiments showed that that some GABAergic neurons were taken "off line" during the seizure-like stimulation, and this was likely protective for them. In addition to protecting GABAergic cells, NMDA blockade protected granule cells that would otherwise die within 24 h, with the hallmarks of apoptosis, in untreated animals.

Conclusion

The immature brain can be harmed by severe seizures in a model-, convulsant-, age-, and pathway-dependent manner. Seizure-induced cell death in the immature brain can occur as a result of intense neuronal firing in the absence of

systemic changes[49,72,73] and can have lasting effects on behavior including learning and memory.[7,72,87,95,123] Furthermore, because most neurons are postmitotic, recovery is limited, and seizure-induced damage experienced early in life may have deleterious consequences that extend well into maturity.

The damage produced by PPS stimulation in the immature free-moving rat demonstrates a reproducible pattern of damage to both principal neurons and subclasses of inhibitory neurons. The damage can resemble mesial temporal lobe sclerosis.[65] Hyperthermia-induced seizures produce MRI abnormalities, and, in a pattern and evolution that is reminiscent of human cases.[12,13,124] The lithium-pilocarpine model, in 2- and 3-week-old rats produces hippocampal damage (predominating in CA1 at P14) with mossy fiber sprouting and extrahippocampal damage.[72,79] Both the hyperthermia-induced model, and the lithium-pilocarpine model, can lead to the development of spontaneous recurrent seizures.[72,87]

The developmental models of SE reveal highly age- and model-dependent processes in the immature brain that need to be better understood through basic studies. Even in the absence of cell death, SE can have adverse effects on brain development. Pursuing new animal models will be important to accurately reproduce the spectrum of severe seizures experienced by children.[1,21,125] As basic mechanisms of SE-induced cell death, synaptic reorganization, and developmental disruption are revealed, translational research seeking clinically useful neuroprotectants and/or antiepileptogenic drugs will likely progress.

We are beginning to understand the pathophysiology of SE in the adult, but these mechanisms may be age-dependent as well, and we still have little information on the ontogeny of SE. Animal studies have revealed that receptor trafficking during seizures can complicate treatments for convulsions and electrographic seizures.[47] Additional biochemical and molecular studies designed to understand the transition to self-sustaining seizures in developmental models of SE are needed. Determining whether receptor trafficking is related to this transition, and eventual refractoriness of seizures, will be clinically important. These types of studies would also be helpful to determine whether monotherapy or polytherapy is best suited for SE in children.

Developing anticonvulsants that terminate seizures early is important, but studies showing that commonly used anticonvulsants can themselves produce neuronal loss complicates the picture considerably.[104,106] Enhancing inhibition, and blocking excitation, during critical periods of synaptogenesis can block trophic support that determines synapse and cell survival. The pharmacologic challenge is made even more complex due to the sliding time window of vulnerability across brain regions.[8] Some clinical studies have made indirect connections to AED therapies and induced apoptosis.[126] Many, but not all,[111] AEDs can trigger neuronal apoptosis in specific brain regions at specific ages, especially in neonates and infants. Any clinical response to SE in the young has to take into account the delicate balance between the danger of seizures and the dangers of treatment.

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Disclosure

None of the authors have any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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